
DETAILED DESCRIPTION

[Detailed Description of the Invention]
[Industrial Application]
This invention relates to the new bulking agent for liquid chromatography.
[Description of the Prior Art]
The interest about the matter [optical activity in recent years] is increasing remarkably. This is an optically active compound in which each of the amino acid which is a biogenic substance, saccharides, protein, nucleic acids, etc. has an asymmetric center, and it is because it came to be recognized strongly that these optically active compounds are carrying out important work to all the scenes of a life process. Moreover, recently, development of a ferroelectric liquid crystal etc. is also briskly performed using the property which an optically active compound has. And although the optical isomer to the optically active compound made into the object is usually simultaneously obtained when compounding chemically and obtaining these optically active compounds, it considered as the means which carries out separation analysis of these, and the approach using liquid chromatography has spread.
The following three approaches are learned as an approach liquid chromatography separates and analyzes an optical isomer. Namely, a primary method derivatizes the target compound using an optical activity reagent, and is a diastereomer. The approach of the approach and the second approach of separating with the usual distribution adsorption column etc. adding a reagent [optical activity / mobile phase / of liquid chromatography], making form a complex between the compounds made into the object, and separating and the third approach are the approaches of separating using the interaction of the compound and stationary phase which embellish a stationary phase with an optical activity reagent, and are made into the object.
Furthermore, the thing embellished with low molecular weight compounds, such as ** phenylglycine, and a valine, naphthyl ethyl urea, or the derivative of those when the optical activity stationary phase used for the third approach was divided roughly.
** What was embellished with the compound which has inclusion ability, such as cyclodextrin and crown ether.

** What was embellished with protein, such as cow serum albumin, an ovomucoid, and an alpha1-acidity glycoprotein.
** What was embellished with the copper complex of amino acid, such as a proline and a hydroxyproline.
** is raised.
[Problem(s) to be Solved by the Invention]
However, there are all following problems in the approach above-mentioned [these]. Time amount may be taken, a by-product may arise for racemization of the optical-activity reagent moreover used, and a reaction, and the approach of derivatizing the compound made into the first object cannot necessarily be said to measurement of optical purity as the optimal approach. Moreover, the method of adding the second additive is not suitable for the object which isolates a sample preparatively, and there is a problem also in profitability. Although it can say that the separation method using the third optical activity stationary phase has conquered the fault which the second approach has for a start [aforementioned], the stationary phase using the low molecular weight compound of ** has the fault that the range of the compound which demonstrates optical discernment ability only with the solvent which made the hexane the subject in many cases, and is divided is also restricted. ** The target compound is three-dimensional, and the constraint about being bulky is large, and the class of solvent used is restricted to water, and an alcoholic system and a perchloric acid water solution, and further, the stationary phase using the compound which has inclusion ability also has a fault -- satisfying division is not

obtained -- if it does not analyze at low temperature. ** Although the stationary phase using protein can divide the compound of the comparatively large range, since protein is used, only degradation of a thing stationary phase with the difficult activity of an organic solvent being comparatively quick and very little **** have a fault -- division is not performed. And the stationary phase using the copper complex of ** has that copper salt must be added to a solvent, and a fault -- the compound which can be divided is restricted to an amino acid derivative etc..

The object of this invention is to have large applicability and provide a Measuring condition with the very stable bulking agent for liquid chromatography with little constraint to the bulking agent which solved the trouble accompanying the above conventional techniques, i.e., the compound made into the object.

[The means for solving a technical problem]

this invention person etc. found out that this bulking agent was what can attain the above-mentioned object, as a result of repeating wholeheartedly research of the bulking agent for liquid chromatography which fixed the vancomycin paying attention to the property to recognize the specific peptide chain which a vancomycin has. Having optical recognition ability was confirmed like [the bulking agent which fixed these] BAIKO mycin by the antibiotic group which furthermore has a vancomycin, similar structure, and an operation mechanism, or its derivative.

That is, the summary of this invention has a vancomycin group antibiotic or its derivative in the bulking agent for liquid chromatography characterized by insoluble support coming to be fixed at a solvent through covalent bond. The bulking agent for liquid chromatography of this invention is called the bulking agent of this invention below.

A vancomycin is the antibiotic produced by streptomyces cage en TARISU, and is effective in gram positives, such as Staphylococcus and a streptococcus. As the operation mechanism, it combines with the D-alanyl-D-alanine radical of a peptidoglycan precursor specifically, and checking the biosynthesis of the peptidoglycan of a bacterial cell wall is known.

A vancomycin, a ristocetin, AKUCHI noy gin, ABOPARUSHIN, AKUTAPURANIN, TEIKO mycin A2, etc. can be illustrated as a vancomycin group antibiotic. Moreover, the compound which esterified acylation, alkylation, the aralkyl-ized compound, or the carboxyl group for the hydroxyl group or amino group can be illustrated as those derivatives.

As the solvent used for the bulking agent of this invention Besides ****, for example, a methanol, ethanol, isopropyl alcohol, Alcohols, such as ethylene, THF, dioxane, ethyl ether, Ketones, such as ether, such as isopropyl ether, an acetone, and a methyl ethyl ketone Hydrocarbons, such as a hexane, a heptane, an octane, benzene, toluene, a xylene, and a cyclohexane Halogenated hydrocarbon, such as chloroform, ethylene chloride, and a dichloroethane Amides, such as ester, such as methyl acetate and ethyl acetate, dimethylformamide, and dimethylacetamide, dimethyl sulfoxide, an acetonitrile, etc. may be used, and a mixed solvent system is [these may be single or] sufficient as them. Moreover, the additive of these salts can also be used. Thus, compared with the conventional bulking agent, it is one of the descriptions of the bulking agent of this invention that a solvent has little constraint.

Next, about the support used for this invention, if insoluble to the above solvents usually used for liquid chromatography, there will be especially no limit. For example, the support of inorganic systems, such as support of synthetic macromolecule systems, such as support of naturally-ocurring-polymers systems, such as a cellulose and agarose, polystyrene, polymethacrylate, polyvinyl alcohol, and polyacrylamide, a silica, an alumina, and a zirconia, etc. can be illustrated.

There is especially no limit about the approach of fixing a vancomycin group antibiotic or its derivative in a solvent at insoluble support. Namely, a joint radical (a spacer is called below.) can be introduced into reactant functional groups, such as a hydroxyl group which exists in a vancomycin group antibiotic or its derivative, an amino group, and a carboxyl group, the reactant functional group which exists in support, or support, and it can carry out by making it combine with the functional group which exists in the spacer. What is necessary is for there to be especially no limit also about the spacer introduced into support, and just to have the reactant functional group in both ends. As the hydroxyl group which exists in a vancomycin group antibiotic or its derivative, and a combinable functional group, an epoxy group, a carboxyl group, a sulfonic group, a halogen radical, etc. are raised, and these functional groups should just exist in support or a spacer. As the amino group which exists in a vancomycin group antibiotic or its derivative, and a combinable functional group, an epoxy group, a carboxyl group, a sulfonic group, a halogen radical, an aldehyde group, etc. are raised, and these

functional groups should just exist in support or a spacer. When the functional group which exists in a vancomycin group antibiotic or its derivative is a carboxyl group, as a functional group in which they and association are possible, the amino group, an epoxy group, a hydroxyl group, a hydrazino radical, a thiol group, etc. are raised, and these should just exist in support or a spacer.

It faces carrying out covalent bond of a vancomycin group antibiotic or its derivative to the spacer combined with support or support, and according to the functional group which support or a spacer has, if required, it can carry out under a suitable solvent using a catalyst, a reaction reagent, etc. suitably. As a catalyst, when functional groups are acids, such as a hydrochloric acid, and a sodium carbonate or a sodium hydrogencarbonate, and alkali is mainly an epoxy group, for example, it is used. Moreover, it is a reaction reagent. When a functional group is a carboxyl group, the combination of **, for example, N-hydroxysuccinic acid imide, and dicyclohexyl carboxy imide A functional group Moreover, a carboxyl group, [a condensing agent like dicyclohexyl carboxy imide or a 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide] In the case of the amino group or a hydrazino radical, moreover, 1 and 1'-carbonyldiimidazole and 2-fluoro-1-methyl pyridinium, It can illustrate [that a condensing agent like p-toluene sulfonate is used when a functional group is a carboxyl group, the amino group, a hydrazino radical, or a hydroxyl group, and a reducing agent like hydrogenation cyano boron sodium is used when a functional group is a formyl group, etc. and]. Moreover, what is necessary is just to use the solvent which can dissolve water, dimethylformamide and a vancomycin group antibiotic like dimethyl sulfoxide, or its derivative as a solvent. Furthermore, water can also be used as a phosphoric acid or the acetic-acid buffer solution, and it is also possible to add and use mineral, such as a sodium chloride and a sodium sulfate.

Although there is not necessarily no limit about a reaction condition with the spacer combined with a vancomycin group antibiotic or its derivative, support, or support, it is appropriate to carry out in the following range generally.

the weight ratio of the support which combined support or a spacer, a vancomycin group antibiotic, or its derivative -- 1:0.01-2.00 -- 0 degree C - 80 degrees C of 4 degree-C-40 degree-C; reaction time of 1:0.05-0.2; reaction temperature are 2 - 12 hours preferably for 1 to 72 hours. There are no requirements special also about the after treatment after a reaction, and they are suitably carried out by approaches usually performed, such as washing according to **.

Thus, the bulking agent of obtained this invention can also be used at the elevated temperature of 60 degrees C - 80 degrees C which has the possibility of denaturation in the bulking agent which embellished the stationary phase with protein.

[Example]

Next, a typical example is shown and the example of analysis using the bulking agent of this invention and it which were manufactured by the following approaches is explained still more concretely. However, these are examples of the bulking agent of this invention, and it cannot be overemphasized that this invention is not restricted to these at all.

Example 1 Ring breakage denaturation of the epoxy group of the epoxy group content gel copolymer obtained from glycidyl methacrylate and ethylene glycol dimethacrylate was carried out with water, and, in addition to 10ml of 0.1-mol sodium-hydroxide water solutions containing vancomycin 0.1g, overnight neglect of the gel (epoxy group: per desiccation gel 1g 0.3 millimols) 1.0g which introduced the epoxy group by epichlorohydrin further was carried out after 24-hour churning by 40-degree Centigrade at 4 times Centigrade. Gel was ****(ed) and an acetic-acid water solution and water washed 1%. In this way, the obtained bulking agent for liquid chromatography was confirmed when making 15mg of vancomycins support per desiccation gel 1g analyzed an unreacted raw material with high performance chromatography.

Example 2 In addition to 10ml of 0.1-mol sodium-hydroxide water solutions containing vancomycin 0.1g, overnight neglect of the gel (epoxy group: per desiccation gel 1g 0.3 millimols) 1.0g which introduced the epoxy group into porous silica gel (Wako Pure Chem make: WAKOGERU LC-10K) by 3-glycidoxy propyl TORIMEETOKISHI silane was carried out after 24-hour churning by 40-degree Centigrade at 4 times Centigrade. Gel was ****(ed) and an acetic-acid water solution and water washed 1%. In this way, the obtained bulking agent for liquid chromatography was confirmed when making 15mg of vancomycins support per desiccation gel 1g analyzed an unreacted raw material with high performance chromatography.

Example 3 Ring breakage denaturation of the epoxy group of the epoxy group content gel copolymer obtained from glycidyl methacrylate and ethylene glycol dimethacrylate is carried out with water. Furthermore, an epoxy

group is introduced with 1,4-butanediol diglycidyl ether. Subsequently, gel (amino group: per desiccation gel 1g 0.2 millimols) 1.0g which was made to act with ammonia and was obtained is added to 5ml (pH 6.5) of 0.1-mol dibasic-sodium-phosphate buffer solutions containing vancomycin 0.2g. Subsequently, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide about 20mg is added, and it ice-cools. It was made to react under a shaking for 3 hours. Subsequently, gel was ****(ed) and 1M sodium chloride water solution and water washed. In this way, the obtained bulking agent for liquid chromatography was confirmed when making about 20mg of vancomycins support per desiccation gel 1g analyzed an unreacted raw material with high performance chromatography.

Example 4 Ring breakage denaturation of the epoxy group of the epoxy group content gel copolymer obtained from glycidyl methacrylate and ethylene glycol dimethacrylate is carried out with water, an epoxy group is introduced with further 1 and 4-butanediol diglycidyl ether, and, subsequently it is water. In addition to 10ml of 0.1-mol sodium-hydrogencarbonate water solutions which contain vancomycin 0.1g in gel 2.0g which made 1 and 1'-carbonyldiimidazole act on the gel which carried out ring breakage denaturation of this epoxy group, and activated the hydroxyl group by the activity carbonyl group, overnight neglect was carried out after 4-hour churning by 40-degree Centigrade at 4 times Centigrade. ****(ed) gel, 10ml (pH 7.5) of 1M tris hydrochloric-acid buffer solutions was made to suspend for 2 hours, and 1M sodium chloride water solution and water washed after that. In this way, the obtained bulking agent for liquid chromatography was confirmed when making 15mg of vancomycins support per desiccation gel 1g analyzed an unreacted raw material with high performance chromatography.

Example 5 In addition to 10ml (pH 8.0) of 0.1-mol dibasic-sodium-phosphate buffer solutions which contain ristocetin 0.1g for gel (aldehyde group: per gel 1ml 0.05 millimols) 2.0g which introduced the epoxy group into porous cellulose gel (Chisso [Corp.] make: SERURO fine A-3) by the bottom epichlorohydrin of 20% sodium-hydroxide existence, and sodium periodate was subsequently made to act and introduced the aldehyde group, hydrogenation cyano boron sodium 20mg was added further, and it agitated by 30-degree Centigrade for 12 hours. Gel was ****(ed) and 1M sodium chloride water solution and water washed. In this way, the obtained bulking agent for liquid chromatography was confirmed when making 4mg of ristocetins support per gel 1ml analyzed an unreacted raw material with high performance chromatography.

Example 6 It is water about the epoxy group content gel copolymer obtained from glycidyl methacrylate and ethylene glycol dimethacrylate. Carry out ring breakage denaturation of this epoxy group, and an epoxy group is further introduced with 1,4-butanediol diglycidyl ether. Subsequently, the acetylation object according gel (amino group: per desiccation gel 1g 0.2 millimols) 1.0g which was made to act with ammonia and was obtained to a vancomycin 0.2g acetic anhydride (whenever [hydroxyl-group or amino group acetylation-] was guessed from the about 40%;NMR spectrum.) In addition to 5ml of included 0.2M potassium-phosphate water solutions, subsequently 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide about 20mg was added, and it ice-cooled, and was made to react under a shaking for 3 hours. Subsequently, gel was ****(ed) and 1M sodium chloride water solution and water washed. In this way, the obtained bulking agent for liquid chromatography was confirmed when making about 20mg of acetylation vancomycins support per desiccation gel 1g analyzed an unreacted raw material with high performance chromatography.

Application 1 The bulking agent for liquid chromatography obtained in the examples 1-6 was filled up with slurry method at the with a bore die length [50mm die length of 8mm] column made from stainless steel, using the obtained column, the CBZ-alanine was separated as one example of an amino acid derivative, and ketoprofen was separated as one example of drugs. The Measuring condition is as follows.

Eluate: 0.1M Sodium phosphate buffer solution (pH 7.0)

Elution rate: 1.0 ml/min Detector: Ultraviolet spectroscopy photometer 254nm This result is shown in a table 1 and a table 2.

表　　1

充填剤の種類	保持時間(分) CBZ－アラニン		分離係数
	Dフォーム	Lフォーム	α
実施例1	7.2	5.0	1.73
実施例2	5.8	5.4	1.11
実施例3	4.9	4.4	1.2
実施例4	5.4	3.2	2.83
実施例5	13.0	6.4	2.5
実施例6	8.0	7.4	1.11

・CBZ：ベンジルオキシカルボニル

表　　2

充填剤の種類	保持時間(分) ケトプロフェン		分離係数
	Rフォーム	Sフォーム	α
実施例1	11.0	14.0	1.35
実施例2	7.8	9.0	1.22
実施例3	8.5	10.5	1.33
実施例4	9.0	12.6	1.55
実施例5	10.8	17.3	1.78
実施例6	14.0	15.5	1.13

[Effect of the Invention]
The bulking agent for liquid chromatography with which a solvent comes to fix the vancomycin group antibiotic offered by this invention or its derivative at insoluble support through covalent bond has little denaturation and possibility of degradation, and there are compared with the very stable bulking agent of the former [constraint / of a solvent]. [few] Furthermore, it is possible to carry out separation analysis of the various optical isomers, such as amino acid, an amino acid derivative, an oxy acid derivative, and drugs, with high performance chromatography by using this bulking agent also about the compound made into the object of separation.

[Translation done.]

CLAIMS

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(57) [Claim(s)]
[Claim 1] The bulking agent for liquid chromatography with which a vancomycin group antibiotic or its derivative is characterized by insoluble support coming to be fixed at a solvent through covalent bond.

[Translation done.]